

Reliable generation of glial enriched progenitors from human fibroblast-derived iPSCs.

Journal:	Stem Cell Res
Publication Year:	2021
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PubMed link:	34274773
Funding Grants:	Human-induced pluripotent stem cell-derived glial enriched progenitors to treat white matter stroke and vascular dementia.

Public Summary:

White matter stroke (WMS) occurs as small infarcts in deep penetrating blood vessels in the brain and affects the regions of the brain that carry connections, termed the subcortical white matter. WMS progresses over years and has devastating clinical consequences. Unlike large grey matter strokes, WMS disrupts the axonal architecture of the brain and depletes astrocytes, oligodendrocyte lineage cells, axons and myelinating cells, resulting in abnormalities of gait and executive function. An astrocytic cell-based therapy is positioned as a strong therapeutic candidate after WMS. In this study we report, the reliable generation of a novel stem cell-based therapeutic product, glial enriched progenitors (GEPs) derived from human induced pluripotent stem cells (hiPSCs). By transient treatment of hiPSC derived neural progenitors (hiPSC-NPCs) with the small molecule deferroxamine, a prolyl hydroxylase inhibitor, for three days hiPSC-NPCs become permanently biased towards an astrocytic fate, producing hiPSC-GEPs. In preparation for clinical application, we have developed qualification assays to ensure identity, safety, purity, and viability of the cells prior to manufacture. Using tailored q-RT-PCR-based assays, we have demonstrated the lack of pluripotency in our final therapeutic candidate cells (hiPSC-GEPs) and we have identified the unique genetic profile of hiPSC-GEPs that is clearly distinct from the parent lines, hiPSCs and iPSC-NPCs. After completion of the viability assay, we have established the therapeutic window of use for hiPSC-GEPs in future clinical applications (7 h). Lastly, we were able to reliably and consistently produce a safe therapeutic final product negative for contamination by any human or murine viral pathogens, selected bacteria, common laboratory mycoplasmas, growth of any aerobes, anaerobes, yeast, or fungi and 100 times less endotoxin levels than the maximum acceptable value. This study demonstrates the reliable and safe generation of patient derived hiPSC-GEPs that are clinically ready as a cell-based therapeutic approach for WMS.

Scientific Abstract:

White matter stroke (WMS) occurs as small infarcts in deep penetrating blood vessels in the brain and affects the regions of the brain that carry connections, termed the subcortical white matter. WMS progresses over years and has devastating clinical consequences. Unlike large grey matter strokes, WMS disrupts the axonal architecture of the brain and depletes astrocytes, oligodendrocyte lineage cells, axons and myelinating cells, resulting in abnormalities of gait and executive function. An astrocytic cell-based therapy is positioned as a strong therapeutic candidate after WMS. In this study we report, the reliable generation of a novel stem cell-based therapeutic product, glial enriched progenitors (GEPs) derived from human induced pluripotent stem cells (hiPSCs). By transient treatment of hiPSC derived neural progenitors (hiPSC-NPCs) with the small molecule deferroxamine, a prolyl hydroxylase inhibitor, for three days hiPSC-NPCs become permanently biased towards an astrocytic fate, producing hiPSC-GEPs. In preparation for clinical application, we have developed qualification assays to ensure identity, safety, purity, and viability of the cells prior to manufacture. Using tailored q-RT-PCR-based assays, we have demonstrated the lack of pluripotency in our final therapeutic candidate cells (hiPSC-GEPs) and we have identified the unique genetic profile of hiPSC-GEPs that is clearly distinct from the parent lines, hiPSCs and iPSC-NPCs. After completion of the viability assay, we have established the therapeutic window of use for hiPSC-GEPs in future clinical applications (7 h). Lastly, we were able to reliably and consistently produce a safe therapeutic final product negative for contamination by any human or murine viral pathogens, selected bacteria, common laboratory mycoplasmas, growth of any aerobes, anaerobes, yeast, or fungi and 100 times less endotoxin levels than the maximum acceptable value. This study demonstrates the reliable and safe generation of patient derived hiPSC-GEPs that are clinically ready as a cell-based therapeutic approach for WMS.

